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14. ABSTRACT
Acquiring a spinning disk confocal microscope has revolutionized the instructional training and research capabilities that faculty at Howard University and other local Colleges and Universities can perform. This technology now allow us to observe very rapid biological events and provide a new level of both spatial and temporal investigation into our imaging capabilities with the final result being a dramatic enhancement in our instructional and research abilities. Researchers across the University, in the Department of Biology and within the School of Engineering and College of Medicine, have benefited from the acquisition of this technology and are

15. SUBJECT TERMS
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Report Title

Final Report: Acquisition of a Spinning Disk Confocal Microscope to Enhance Research and Teaching Capabilities in the STEM Fields at Howard University

ABSTRACT

Acquiring a spinning disk confocal microscope has revolutionized the instructional training and research capabilities that faculty at Howard University and other local Colleges and Universities can perform. This technology now allow us to observe very rapid biological events and provide a new level of both spatial and temporal investigation into our imaging capabilities with the final result being a dramatic enhancement in our instructional and research abilities. Researchers across the University, in the Department of Biology and within the School of Engineering and College of Medicine, have benefited from the acquisition of this technology and are utilizing the microscope every day for the individual research pursuits. In addition, this state-of-the-art imaging system has begun to be incorporated into the course offerings at both the undergraduate and graduate levels starting the 2014-2015 academic year. The instrument has also been utilized by Trinity University faculty to educate their students. By instructing undergraduate students on a state-of-the-art microscope such as the spinning disk, we will strengthen our discipline and improve the training of these students in the STEM fields.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received	Paper

TOTAL:

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received	Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

1) Identifying new players in meiosis using C. elegans depleted of the meiotic inhibitory kinase WEE-1.3.
Ruby Boateng, Jessica Nesmith, Andy Golden, and Anna K Allen
Department of Biology, Howard University, Washington, D.C.
Conference: 2014 American Society for Cell Biology Meeting, December 6-10, 2014
Poster Presentation

Abstract:
Meiosis is the specialized cell cycle by which the haploid gametes, oocytes and sperm, are produced. Errors in meiosis can result in serious issues such as infertility, birth defects or tumorigenesis. Meiosis is controlled via dueling regulatory phosphorylation events on the cyclin-dependent kinase (Cdk) component of maturation promoting factor (MPF). The highly conserved Wee1/Myt1 family of kinases places inhibitory phosphorylations on Cdk that are necessary to keep MPF inactive and thus regulate the progression of meiosis. Despite many years of study, in multiple animal systems, our understanding of meiosis is still incomplete. The studies described here in Caenorhabditis elegans are aimed to increase our knowledge regarding players in the meiotic pathway.

In C. elegans it was previously shown that depletion of the Myt1 ortholog, WEE-1.3, results in precocious oocyte maturation and infertility (Burrows et al., 2006). We further characterized the precocious oocyte maturation phenotype observed upon WEE-1.3 depletion to better grasp why the oocytes are fertilization-incompetent. We will demonstrate here that WEE-1.3-depleted proximal germlines have begun to transcribe embryonic genes and the precocious oocytes inappropriately express proteins in patterns normally found in embryos. Secondly, we performed an RNA interference (RNAi) suppressor screen of the infertility phenotype exhibited upon WEE-1.3 depletion to identify new players involved in the meiotic pathway. We found 44 genes, that when co-depleted along with WEE-1.3, restore fertility to the animals. Many of these genes previously had no known role in reproduction or meiosis. Finally, we will report on the initial studies being conducted on one specific suppressor, ETR-1. ETR-1 is an RNA-binding protein that was previously characterized as being muscle-specific in the worm (Milne and Hodgkin, 1999). However, homologs in other organisms, including humans, have reproductive roles and mutations in some of the homologs have been linked to fertilization defects. Our data indicates a novel role for ETR-1 in the somatic gonad that ultimately influences oocyte maturation. We are actively investigating the mechanism by which depletion of ETR-1 suppresses WEE-1.3 depletion.

As our studies of ETR-1 are alluding to, many of the genes identified in the RNAi suppression screen have the potential to be unidentified players in both the meiotic and mitotic cell cycles. Thus these studies are providing valuable input into how the cell cycle is appropriately regulated.

2) Absence of mitochondrial SOD2 in Drosophila induces a novel peroxidase, which plays an essential role during adult wing maturation.
Dondra Bailey, Sanjay Nag, Mohammed Basar, Atanu Duttaroy.
Department of Biology, Howard University, Washington, DC.
Conference: 56th Annual Drosophila Research Conference, March 4-8, 2015
Poster Presentation

Abstract:
SOD2 (superoxide dismutase 2) is a nuclear encoded protein, which catalyzes the conversion of superoxide radicals into hydrogen peroxide in the mitochondria. A null mutant for the Sod2 gene, Sod2n283 survives a very short adult life span like the Sod2 KO mice. Should this high flux of mitochondrial ROS in Sod2n283 facilitate the activation of novel gene(s) most likely through retrograde mechanism? Gene array analysis revealed various transcriptomes are altered significantly in their expression pattern in the Sod2n283. Notably among them is the Drosophila gene CG5873, which was upregulated ~40 fold in Sod2n283. CG5873 carries a highly conserved domain classified as the animal peroxidase superfamily. Interestingly, both the insertion mutant as well as ubiquitous ablation of CG5873 with RNA RNAi shows a collapsed and wrinkled wing phenotype in adults. Our analysis with early wing specific GAL4 drivers, revealed no associated defects in wing morphology when combined with the CG5873IR, which led us to conclude that CG5873 is not required during wing development. On the other hand, ablation of CG5873 during adult wing maturation shows collapsed wing phenotype, suggesting the requirement of this peroxidase function during tissue remodeling process of adult wing maturation. .

3) ETR-1, an ELAV-type RNA binding protein, may play a role in regulating germline apoptosis in Caenorhabditis elegans
Ruby Boateng (graduate student) and Anna Allen
Department of Biology, Howard University, Washington, DC.
Conference: Howard University Research Day 2015, April 16, 2015

Abstract:
Background: ETR-1, an ELAV-type RNA binding protein (RBP), is a muscle protein in Caenorhabditis elegans whose depletion results in reduced brood size in specific backgrounds, paralyzed arrested elongation two-fold and lethality. Human homologs of ETR-1 have been implicated in myotonic muscular dystrophy when mutated. Recently, ETR- 1 was identified in a large-scale C. elegans RNAi suppressor screen for genes that when co-depleted with WEE-1.3 resulted in a restoration of fertility from the otherwise very severe infertile phenotype exhibited upon wee-1.3 (RNAi). This led us to question whether ETR-1 has previously overlooked roles in the germline, particularly in

regulating apoptosis and fertility of this nematode. Methods: C. elegans techniques such as standard brood analysis, RNA interference (RNAi), CRISPR-Cas9 genome editing, and immunohistochemistry are utilized. Results: We will demonstrate that depletion of ETR-1 alone results in reduced fertility and is due to an increase in germline apoptosis. We are utilizing the CRISPR- Cas9 technology to generate genome-edited lines where green fluorescent protein (GFP) endogenously tags specific isoforms of ETR-1. This will allow us to determine both the temporal and spatial expression pattern of ETR-1, including subcellular localization and specific role(s) of the multiple isoforms by ETR-1. Conclusions: Identifying the mechanism will provide knowledge regarding an RNA binding protein with a novel function within the meiotic cell cycle. Furthermore, understanding the role of etr-1 will be used to help understand the role of CUG-BP in muscular dystrophy, as well as the causes of infertility that is associated with a failed cell cycle.

4) Uncovering putative interactions of the inhibitory kinase WEE-1.3 in C. elegans
Lourds Michelle Fernando and Anna Allen
Department of Biology, Howard University, Washington, DC. April 16, 2015
Conference: Howard University Research Day 2015

Abstract:
Background: The Ceanorhabditis elegans Myt-1 ortholog, WEE-1.3, is an inhibitory kinase that regulates meiosis by providing inhibitory phosphorylations on CDK-1, a component of Maturation Promoting Factor (MPF). Inactivation of MPF leads to oocyte meiotic cell cycle arrest at prophase I, a process needed for the developmental competency. Depletion of WEE-1.3 causes precocious oocyte maturation and generates fertilization-incompetent oocytes. A large RNAi screen by Allen et al. identified 44 genes that when co-depleted with wee-1.3 (RNAi) suppressed the wee-1.3(RNAi) infertility phenotype. Elucidating potential interactions between WEE- 1.3 and the identified suppressors may lead to identifying novel regulators of the cell cycle. To begin to determine mechanism, we have asked whether the down regulation of individual suppressors via RNAi alters the previously characterized WEE- 1.3 localization pattern. Methods: RNA interference, standard brood analysis and confocal live imaging. Results: The live imaging data indicates two of the identified suppressors, snr- 1 and cdk-1, show alterations in the localization of WEE-1.3. Animals depleted of SNR-1 show aberrant nuclear expression of WEE-1.3 in embryos and cdk-1(RNAi) animals completely lack WEE-1.3 expression in embryos. Conclusion: SNR-1 is a snRNP that may be involved in perinuclear localization of WEE-1.3 in developing embryos. Absence of CDK-1 affecting WEE-1.3 localization implies a potential reciprocal relationship where CDK-1 can regulate WEE-1.3 in a comparable manner to how WEE-1.3 regulates CDK-1. Further analysis of WEE- 1.3 localization patterns of the remaining suppressors will aid in elucidating interactions of WEE-1.3.

5) Characterization of a novel role for ETR-1 in reproduction
Ruby Boateng (graduate student), Andy Golden, and Anna Allen
Department of Biology, Howard University, Washington, DC.
Conference: 20th International C. elegans Meeting, June 24-28, 2015

Abstract:
ETR-1, an ELAV-Type RNA-binding protein, is canonically known for its involvement in muscle development, however we have identified novel roles for this protein in both the somatic and germline cells of the C. elegans gonad. RNAi depletion of ETR-1 results in animals paralyzed at the two-fold (PAT) embryonic stage, indicating a failure in normal muscle development, and in a reduced brood compared to control depleted animals, indicating a reproductive role. We hypothesized that the reduced brood size might potentially be due to decreased germ cell numbers, reduced ovulation rate and/or an increase in apoptosis. The number of germ cells and ovulation rate were found to be similar in ETR-1- and control-depleted animals, however only ETR-1-depleted animals exhibit an increased number of apoptotic cells. Interestingly, a recent RNAi based suppressor screen by Allen et. al. (2014) has shown that co-depletion of ETR-1 and WEE-1.3 (an essential regulator of the meiotic cell cycle and oocyte maturation) restores fertility to the otherwise sterile wee-1.3(RNAi) animals. We investigated this further using a somatic RNAi defective worm strain and determined that this ability of ETR-1 to suppress is dependent on ETR-1 being depleted in the soma. ETR-1 depletion only in the germline also resulted in a brood size that was significantly different from its control, uncovering a novel role for ETR-1 in the germline. Further supporting this ETR-1 is expressed in both the somatic muscle sheath cells and the germ cells of the hermaphrodite and male gonads. We hypothesize that these roles may be controlled by the different isoforms of ETR-1. To address this, we are utilizing CRISPR-Cas9 technology to generate genome-edited lines in which green fluorescent protein (GFP) endogenously tags specific isoforms of ETR-1. Ultimately, these studies will deepen our understanding of the role of ETR-1 within both the somatic and germline tissue. This increased understanding will benefit the reproductive biology field and add to our knowledge of the role of human ETR-1 homologue, CUG-BP, in muscular dystrophy.

6) Small Compounds Targeting Tyrosine Phosphorylation of RACK1A Protein Confer ABA Hypersensitivity
Mercy Sabila, Ahsan Rahman, Deborah Fadoju, Joanna Akinlosotu, Rachel Darko, Kyaira Ware, Sivanesan Dakshanamurthy, Hemayet Ullah
Department of Biology, Howard University, Washington, DC.
Conference: Plant Biology 2015 on July 26-30th, 2015
Oral Presentation

Abstract:
RACK1 (Receptor for Activated C Kinase 1) is a WD-40 type scaffold protein, conserved in single cell eukaryote yeast to human and plays regulatory roles in diverse signal transduction and stress response pathways. Loss of function mutant in Arabidopsis indicates that

RACK1A regulates diverse environmental stress resistance and developmental pathways through a negative regulation of stress hormone abscisic acid ABA) signaling pathway . It is hypothesized that chemical knock-out, as opposed to genetic knock-out, of RACK1A will provide a functional advantage in protecting plants from diverse stress through the effect of hypersensitivity to the ABA. Site directed mutagenesis studies indicated that the key post-translational modifications like sumoylation at K273 and tyrosine phosphorylation of Y248 residues dictate the RACK1A's potential to interact with other proteins. In order to facilitate the identification of small compounds binding to the functional pocket, the crystal structure of RACK1A protein is deduced at 2.4 A resolution. Deduced crystal structure of RACK1A is used to identify dozens of small compounds that could potentially bind to the Y248 pocket. The compounds could potentially inhibit Y248 phosphorylation and bind to purified recombinant RACK1 protein with a kD value in the micro-molar ranges. The compounds can effectively elicit ABA signaling pathways through activating the ABI5 gene expression as evaluated in the ABA induced seed germination, growth inhibition assays. The effectiveness of the compounds in regulating diverse environmental stress responses are evaluated in different crop plants. Here we present, evidence that the compounds are effective in regulating salt and drought stress responses in wide variety of crop plants including in tomato, beans, and pepper. To allow ease of application to crop plants, the compounds are being formulated as fertilizer additives.

Number of Presentations: 6.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received Paper

TOTAL:

Number of Manuscripts:

Books

Received Book

TOTAL:

Received Book Chapter

TOTAL:

Patents Submitted

Patents Awarded

Awards

- HU ADVANCE-IT Grant, 2014-2015, $15,062, Principal Investigator- Dr. Anna K. Allen "Characterization of a novel role for ETR-1, an RNA-binding protein in C. elegans, in germline development and/or function."

- ASCB/NSF Faculty Research and Education Developmental (FRED) Award to Dr. Anna K. Allen, 2014-2015

- Ruby Boateng – Honorable Mention in the HU Research Day poster presentations for "ETR-1, an ELAV-type RNA binding protein, may play a role in regulating germline apoptosis in Caenorhabditis elegans"

Graduate Students

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Names of Faculty Supported

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: 0.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:..... 0.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):..... 0.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense 0.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:..... 0.00

Names of Personnel receiving masters degrees

NAME

Total Number:

Names of personnel receiving PHDs

NAME

Total Number:

Names of other research staff

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

See Attachment

Technology Transfer

FINAL REPORT: Scientific Progress and Accomplishments

PROJECT TITLE: Acquisition of a Spinning Disk Confocal Microscope to Enhance Research and Teaching Capabilities in the STEM Fields at Howard University

AGREEMENT NUMBER: W911NF-14-1-0073

Principal Investigator: Dr. Anna Allen
Biology Department
Howard University

1- Foreword

This first period of the award was utilized to purchase, install and train investigators and student researchers on the spinning disc confocal microscope. We now have an active cohort of individuals who have been trained on the equipment and are beginning to utilize the equipment to address their specific scientific questions.

2- Statement of Problem Studies

The long-term research objective of the principal investigator's (PI's) laboratory is to identify novel regulators of the cell cycle and to understand the mechanism by which these regulators control the cell cycle. It is assumed that these unknown regulators in our genome ensure that abnormal progression of the cell cycle does not occur. We are currently actively investigating the role for specific members of the ubiquitin-proteasome system (UPS) in cell cycle regulation, specifically in the regulation of the levels of cyclin-dependent kinase 1 (CDK-1). Other laboratories, such as Drs. Duttaroy, Jones and Ullah, are utilizing the microscope to study the effects of oxidative stress in *Drosophila*, to determine the cellular function of scaffold proteins involved in signal transduction pathways in *Arabidopsis thaliana*, and to examine the physiology of GABAergic interneurons in mental health disorders such as depression and drug abuse in order to design better therapeutic drugs.

A secondary objective in obtaining the spinning disk confocal microscope was to enhance the availability of training opportunities to our undergraduate and graduate student population at Howard University. Prior to the purchase of the spinning disk confocal microscope, our instructional capabilities in the area of microscopy were very limited at Howard University. Now we are able to provide our student population, consisting mainly of underrepresented minority students, with an outstanding educational experience that includes state-of-the-art technology like the spinning disk.

3- Summary of Scientific Results

<u>Characterization of a Novel Role for ETR-1 in Reproduction</u>

Thesis work of Ms. Ruby Boateng (Dr. Anna Allen laboratory)

Background - Infertility is a major issue that affects about 10-15% of people in the United States[2,3]. Despite its prevalence (10.9% impaired fecundity in women ages 15-44 and 6.0% infertility in married women ages 15-44) and some of its known causes (i.e. ovulation problems and low sperm count), people are still unable to conceive even when

these causes are treated. In order to overcome infertility, people often seek therapeutics such as in-vitro fertilization (manual fusion of egg and sperm), but in most cases, this treatment fails. This inability to conceive even with all the available treatment options or the fusion of an ideal oocyte and sperm means that there is still a cause(s) of infertility that has yet to be discovered. While the source of these causes remains unidentified, a failed meiotic cell cycle is often held liable.

A complex network of regulatory proteins controls the meiotic cell cycle. This complexity makes it difficult to know the number and function of all of the proteins involved. As a result, problems within specific processes, such as fertilization, are often attributed to the malfunction of the entire cell cycle. A suppressor screen conducted with the *Caenorhabditis elegans* cell cycle inhibitory protein, WEE-1.3, implied a previously unidentified role for the protein ETR-1 in meiosis[1]. This was confirmed when ETR-1 depletion via RNA interference (RNAi; endogenous gene silencing technique) resulted in reduced sterility in C. elegans (Fig 1). *C.elegans* are effective model systems because they have a completely sequenced genome that can easily be manipulated using post-genomic tools such as RNAi. Using *C. elegans* as a model system, the role of ETR-1 in the meiotic cell cycle is being investigated.

Introduction-

ETR-1, an ELAV-Type RNA-binding protein, is canonically known for its involvement in muscle development, however we have identified novel roles for this protein in both the somatic and germline cells of the *C. elegans* gonad. RNAi depletion of ETR-1 results in animals paralyzed at the two-fold (PAT) embryonic stage, indicating a failure in normal muscle development, and in a reduced brood compared to control depleted animals, indicating a reproductive role (Fig. 1). We hypothesized that the reduced brood size might potentially be due to decreased germ cell numbers, reduced ovulation rate and/or an increase in apoptosis. Interestingly, a recent RNAi based suppressor screen by Allen *et. al.* (2014) has shown that co-depletion of ETR-1 and WEE-1.3 (an essential regulator of the meiotic cell cycle and oocyte maturation) restores fertility to the otherwise sterile *wee-1.3(RNAi)* animals. We investigated this further using a somatic RNAi defective worm strain and determined that this ability of ETR-1 to suppress is dependent on ETR-1 being depleted in the soma (Fig. 1). Further supporting this, ETR-1 is expressed in both the somatic sheath of the hermaphrodite and the germ cells of both the hermaphrodite and male gonads (Fig. 2). We hypothesize that germline and somatic roles may be controlled by different isoforms of ETR-1. To address this, we are utilizing

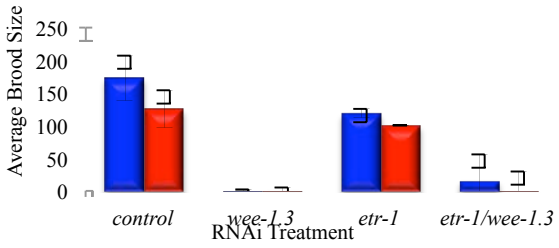

Figure 1: *Fertility Assay:* ETR-1 depletion results in reduced brood size when compared with the control RNAi treatment. Additionally, it suppresses the WEE-1.3 depletion sterility. Blue bars = wild-type; Red bars = RNAi somatic defective.

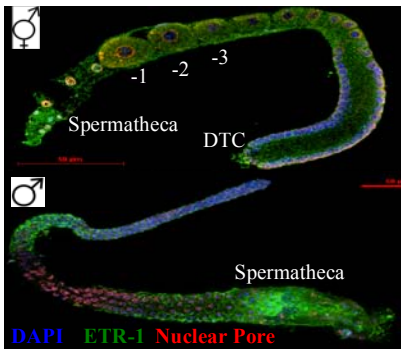

Figure 2: *Immunohistochemistry*: ETR-1 is expressed in the gonads of hermaphrodite and males.

CRISPR-Cas9 technology to generate genome-edited lines in which green fluorescent protein (GFP) endogenously tags specific isoforms of ETR-1. Ultimately, these studies will deepen our understanding of the role of ETR-1 within both the somatic and germline tissue. This increased understanding will benefit the reproductive biology field and add to our knowledge of the role of the human ETR-1 homologue, CUG-BP– implicated myotonic muscular dystrophy[2].

Results and Discussion-

Previous studies on ETR-1 have implicated its somatic role (PAT), however, recent studies have shown that ETR-1 may have a germline role as its depletion results in a reduced brood size (Fig 1). We hypothesized this reduced brood may potentially be due to decreased germ cell numbers, reduced ovulation rate and/or an increase in apoptosis. The gonad size and germ cell numbers of ETR-1- and control-depleted worms were imaged and measured to be similar 24 hours post RNAi treatment (summary in Table 1). The ovulation rate of ETR-1-depleted worms was determined by calculating the number of embryos laid over a 5 hour time period to be similar to that of control depleted worms (Table 1). To examine if the reduced brood size is due to increased apoptosis, ETR-1- and control-depleted worms were stained with acridine orange to visualize apoptotic cells and the numbers of cells quantified via confocal microscopy. ETR-1-depleted worms had increased apoptotic cells when compared to control (Table 1). Additionally, CED-1::GFP (ced-1 engulf pre-apoptotic cells) strains has show increased in ETR-1-depleted worms when compared with control-depleted animals (data not shown). This further confirms that the reduced brood size in ETR-1 depleted worms is due to increased apoptosis.

Summary of Causes of Reduced Brood Size

Experiment	*control*(*RNAi*)	*etr-1*(*RNAi*)
Ovulation Rate	Normal	Normal
Germ cell counts	Normal	Normal
Apoptosis	Normal	Increased

The role of ETR-1 in the germline is strengthened by positive ETR-1 antibody staining in the germline. ETR-1 is expressed in the nucleus of developing embryos, throughout the body muscle, the hermaphrodite gonadal sheath, and within sperm and the oocyte cytoplasm (Fig. 2). These expression patterns confirm localization of ETR-1 to the gonad, both in the soma (sheath) and germline (sperm/oocytes), but do not indicate whether a particular isoform is essential in the soma versus the germline. To distinguish between the isoforms essential in specific tissues, CRISPR/Cas-9 (endogenous genome editing technique) is being utilized to generate genome-edited lines with green fluorescent protein (GFP) endogenous tags. These strains will help determine both the temporal and spatial expression pattern of ETR-1, including subcellular localization and specific role(s) of the multiple isoforms and will allows us to conduct live-imaging of ETR-1.

Conclusion-

This study has shown that ETR-1 has a germline role in addition to its known somatic role. Based on our experiments, we propose three mechanisms by which ETR-1 might be acting: within the meiotic cell cycle, regulating apoptosis and/or during gametogenesis. First, the reduced brood size in ETR-1-depleted animals indicates that ETR-1 may be involved in the meiotic cell cycle. Second, the increased apoptotic cells in ETR-1-

depleted animals suggest that ETR-1 is involved in germline apoptosis. This fact when combined with the gonad sheath expression of ETR-1, implies that ETR-1 might be needed to maintain the integrity of the germline. Finally, ETR-1 also appears to be implicated in gametogenesis as it is expressed throughout the germlines of males and hermaphrodite worms. Taken together our studies show a novel, important role for ETR-1 in the germline and the reproductive capability of *C. elegans*.

Additional research is being conducted in the following areas by various graduate students within the Department of Biology and utilizing the spinning disk:

1- Possible requirement of ROS (Reaction Oxygen Species) signaling in *Drosophila* wing maturation process
   Thesis work of Ms. Dondra S. Bailey (Dr. Atanu Duttaroy laboratory)

2- Nutrient dependent growth regulation appears to be mediated by spargel/dPGC-1 in *Drosophila*
   Thesis work of Mr. Md. Abul Basar (Dr. Atanu Duttaroy laboratory)

3- Specific tissue requirement of dPGC-1/spargel in *Drosophila*
   Thesis work of Mr. Tomilowo Abijo (Dr. Atanu Duttaroy laboratory)

4- Summary of Training

Undergraduate Training:

Currently the microscope is being utilized for informational training purposes and independent research projects in a number of undergraduate courses at Howard University. The PI has utilized the microscope in both her sophomore level Genetics course (BIOL200, Spring 2015) and her upper-level Developmental Biology course (BIOL713, Fall 2014).

In Genetics (BIOL200), approximately 90 enrolled students were able to view the dynamics of a fluorescently tagged *Drosophila melanogaster* protein (Fascin::GFP) in the female ovary during egg development. Students were conducting a pharmacogenetic screen using ovaries dissected from transgenic Fascin::GFP flies and as part of this experiment, we took small groups up to the spinning disk confocal microscope and allowed the students to view the live trafficking and movement of Fascin throughout the Drosophila egg chambers. Prior to the students viewing their samples, they were provided a mini-lecture on fluorescent confocal microscopy, specifically the importance of the spinning disk aspect of this particular scope that allows them conduct real-time, or live, imaging.

Student Comments from BIOL200 (Genetics):

- "This is awesome! I didn't know Howard had stuff that could do this."
- "Can we utilize this microscope for our own individual research?"
- "You mean we have a microscopy that is as good as one at Hopkins?"
- "In what other classes could we get to use the microscope?"

Within Developmental Biology (BIOL713), six students conducted independent research projects on the effects of depleting their gene of interest within the nematode, *Ceanorhabditis elegans*. One manner in which they characterized the effect of their specific gene knockdown was to determine whether there were abnormalities within the germline tissue of transgenic nematodes expressing fluorescent fusion histone proteins (H2B::mCherry) and tubulin proteins (tubulin::GFP). The students were initially trained on the microscope by the PI and graduate teaching assistant, Ms. Ruby Boateng. They learned the theory behind how a spinning disk fluorescent confocal microscope functions and then were able to put that theory into practice by imaging their own individual samples. The spinning disk allowed them to observe over a set time period, how the spatial and temporal expression patterns of the tagged proteins changed under their mutant conditions.

Finally, students from Trinity Washington University, a historically undergraduate women's college with 90% of their student population being members of underrepresented groups, have made trips over to Howard University to be trained on the microscope and to utilize it in their laboratory courses. In February and March of 2015, both Dr. DeBoy and Dr. Moitra from Trinity brought their individual classes over to Howard. Both groups were provided a general overview of confocal microscopy and the capabilities of the spinning disk confocal. For Dr. Moitra's students, they then spent time observing and learning how to image *C. elegans* expressing fluorescently tagged fusion proteins. For Dr. DeBoy's students, they then utilized their time on the microscope to observe fluorescently transformed live *E. coli* and then conducted confocal imaging on stained brain slices from transgenic mice.

Graduate Training:

Currently the microscope is being utilized by a number of graduate students within the Department of Biology for completion of their thesis research. The following students are active users of the microscope and are anticipated to have both future peer-reviewed publications and theses that incorporate large amounts of data gathered through the spinning disk confocal microscope:

Student	Laboratory	Department	Anticipated Thesis Defense
Nujud Almuzaini	Jones	Biology	Fall 2016
Tomilowo Abijo	Duttaroy	Biology	Spring 2016
Dondra Bailey	Duttaroy	Biology	Fall 2015
Md. Abul Basar	Duttaroy	Biology	Spring 2016
Ruby Boateng	Allen	Biology	Spring 2018
Lourds Fernando	Allen	Biology	(first year student)
Nichelle Jackson	Jones	Biology	(first year student)
Anastasia Robinson	Filipeanu	Pharmacology	(first year student)

5- Bibliography

1- Allen et al. *An RNAi-based suppressor screen identifies interactors of the Myt1 ortholog of Caenorhabditis elegans*. G3(Bethesda). 8 Oct. 2014. Print

2- Centers for Disease Control and Prevention. Centers for Disease Control and Prevention,16 Apr. 2015. Web. 30 Apr. 2015.
3- "Infertility." Mayo Clinic. 2 July 2014. Web. 30 Apr. 2015.
4- Milne, C. A., & Hodgkin, J. *ETR-1, a homologue of a protein linked to myotonic dystrophy, is essential for muscle development in Caenorhabditis elegans*. Current Biology. 1999 Nov 4;9(21):1243-6.

6- Appendixes

Included below are e-mail letters from Dr. Deboy and Dr. Karobi who utilized the spinning disk in their courses for Trinity Washington University.

Subject: Thank you!

Date: Thursday, February 26, 2015 6:30:25 AM Eastern Standard Time

From: Cynthia DeBoy

To: Allen, Anna K

Dear Anna,

Thank you so very much for allowing us to use the microscope and helping us so much during our time in your lab. I asked the students to fill out a survey and they all really enjoyed the experience and found it valuable. The success in visualizing the transformed E.coli and hearing about some of the live imaging that can be done, has given me some ideas of how to incorporate a lab into microbiology in the fall. Perhaps, if you are willing, I could discuss at some point whether such a lab would be feasible.

Thank you again! The lab was inspiring!

Cynthia

Cynthia DeBoy, Ph.D.
Clare Boothe Luce Assistant Professor of Biology (Program Chair)
Trinity Washington University
125 Michigan Avenue, NE
Science Building, Room 201
Washington, DC 20017
(202) 884-9257(office)

Subject: Hi and thank you!

Date: Tuesday, April 7, 2015 11:13:55 AM Eastern Daylight Time

From: Karobi Moitra

To: Allen, Anna K

Hi Anna

Thank you so much for hosting us yesterday I really appreciate you taking the time to do this for the students. Sorry that I had to rush off afterwards for the meeting. The students really enjoyed themselves and learned a lot.

Thanks a lot
All the best
Karobi

Karobi Moitra, PhD
Clare Boothe Luce Assistant Professor of Molecular Biology
Trinity Washington University
CAS, Science 203
125 Michigan Ave. NE,
Washington, DC 20017
Email: MoitraK@trinitydc.edu
Phone: 202-884-9225 (Office)